

AMENDMENTS TO THE CLAIMS

The following listing of claims replaces all prior versions, and listings, of claims in this application.

Listing of Claims

1. **(Original)** A method of identifying a transmembrane receptor (TMR) agonist, wherein the TMR agonist (TMRA) is capable of activating TMR signaling while exhibiting reduced TMR internalization over a control compound, comprising the steps of:
 - (a) providing a cell comprising at least one TMR, or a biologically active fragment thereof, wherein the cell further comprises arrestin, or a biologically active fragment thereof,
 - (b) exposing the cell to at least one test compound,
 - (c) measuring the signaling at two or more points in time,
 - (d) measuring the translocation of the TMR at two or more points in time, and (e) quantitatively determining if the internalization is reduced by comparing the TMR internalization in the presence of the test compound to the TMR internalization in the presence of a control compound, and wherein the signaling is activated as compared to TMR signaling in the absence of agonist.
2. **(Original)** The method of claim 1, wherein the TMR is a GPCR.
3. **(Original)** The method of claim 1, wherein the translocation of the TMR is measured by monitoring localization of a detectable molecule bound to the arrestin or the TMR.
4. **(Original)** The method of claim I , wherein signaling is quantitated by measuring an intracellular effector, wherein said effector may be cAMP, cyclic GMP, Calcium, a lipid, phosphatidylinositol, a hydrogen ion, or an ion transport molecule.
5. **(Original)** The method of claim 1, wherein the control compound is a natural ligand or natural agonist.
6. **(Original)** The method of claim 1, wherein the test compound is a modified natural ligand or modified natural agonist.

7. **(Original)** The method of claim 1, wherein the test compound is a known pharmaceutically relevant compound, or is derived from a known pharmaceutically relevant compound.
8. **(Original)** The method of claim 1, wherein the signaling is activated for a longer time period after stimulation by the TMRA than the length of time of activation after stimulation by the control compound.
9. **(Original)** The method of claim 1, wherein the translocation of the TMR is measured by determining the localization in the plasma membrane, pits, endosomes, endocytic vesicles, or cytosol.
10. **(Original)** The method of claim 2, wherein the GPCR is a class A, or class B receptor.
11. **(Original)** The method of claim 2, wherein the GPCR is a μ opioid, R_1AR , $\sim 3_2AR$, or dopamine receptor.
12. **(Original)** The method of claim 1, wherein the translocation of the TMR is measured by visualization of a radioisotope, an epitope tag, an affinity label, an enzyme, a fluorescent group, or a chemiluminescent group attached to the arrestin or the TMR.
13. **(Original)** The method of claim 1, wherein the signaling is measured at the same time as the translocation is measured.
14. **(Original)** The method of claim 1, wherein the cell is exposed to the compound once, and wherein the cell is not exposed a second time to the compound.
15. **(Withdrawn)** A compound discovered by the method of claim 1.
16. **(Original)** The method of claim 1, wherein the TMR is a rat, human, mouse, pig, or primate TMR.
17. **(Original)** The method of claim 1, wherein the method is repeated, and wherein the TMR in the repeat method is from a different species than in the original method.
18. **(Original)** The method of claim 17, wherein a test compound that is a TMRA in the original method is not a TMRA in the repeat method, and wherein the repeat method contains a TMR from a different species.

19. **(Original)** The method of claim wherein the test compound is from a combinatorial library.
20. **(Original)** The method of claim 1, wherein the signaling in the presence of the test compound is approximately equal to or greater than the signaling in the presence of the control compound.
21. **(Original)** The method of claim 1, wherein the method is repeated at different concentrations of compound to yield a dose response curve for the signaling measurement and a dose response curve for the translocation measurement in the presence of the test compound.
22. **(Original)** The method of claim 21, wherein the quantitative determination includes a comparison of the dose response curve for the signaling measurement to the dose response curve for the translocation measurement.
23. **(Original)** The method of claim 21, wherein a second dose response curve for the signaling measurement and a second dose response curve for the translocation measurement are determined in the presence of control compound.
24. **(Original)** The method of claim 23, wherein dose response curve for the translocation measurement in the presence of the test compound is reduced as compared to the dose response curve for the translocation measurement in the presence of the control compound.
25. **(Original)** The method of claim 23, wherein the dose response curve for the signaling measurement in the presence of the test compound is approximately equal to or greater than the dose response curve for the signaling measurement in the presence of the control compound.
26. **(Original)** The method of claim 24, wherein the reduced translocation is determined by a decrease in the Max of the dose response curve for the translocation measurement in the presence of the test compound, as compared to the Max of the dose response curve for the translocation measurement in the presence of the control compound.
27. **(Original)** The method of claim 24, wherein the reduced translocation is determined by an increase in the EC50 of the dose response curve for the translocation measurement in the presence of the test compound, as compared to the EC50 of the dose response curve for the translocation measurement in the presence of the control compound.

28. **(Original)** A method of identifying a modified TMR agonist, wherein the modified TMRA is capable of activating TMR signaling while exhibiting reduced TMR internalization over an unmodified compound, comprising the steps of:

- (a) providing an agonist or ligand of a TMR,
- (b) modifying the agonist or ligand,
- (c) providing a cell comprising at least one TMR, or a biologically active fragment thereof, wherein the cell further comprises arrestin, or a biologically active fragment thereof,
- (d) exposing the cell to a modified agonist(s) or modified ligand(s),
- (e) measuring the signaling at two or more points in time,
- (f) measuring the translocation of the TMR at two or more points in time, and (g) quantitatively determining if the internalization is reduced by comparing the TMR internalization in the presence of the test compound to the TMR internalization in the presence of an unmodified compound, and wherein the signaling is activated as compared to TMR signaling in the absence of agonist.

29. **(Original)** The method of claim 28, wherein the TMR is a GPCR.

30. **(Original)** The method of claim 28, wherein the translocation of the TMR is measured by monitoring localization of a detectable molecule bound to the arrestin or the TMR.

31. **(Original)** The method of claim 28, wherein signaling is quantitated by measuring an intracellular effector, wherein said effector may be cAMP, cyclic GMP, Calcium, a lipid, phosphatidylinositol, a hydrogen ion, or an ion transport molecule.

32. **(Original)** The method of claim 28, wherein the control compound is a natural ligand or natural agonist.

33. **(Original)** The method of claim 32, wherein the natural ligand or natural agonist is a known pharmaceutically relevant compound, or is derived from a known pharmaceutically relevant compound.

34. **(Withdrawn)** A compound discovered by the method of claim 28.

35. **(Original)** The method of claim 28, wherein the signaling is activated for a longer time period after stimulation by the TMRA than the length of time of activation after stimulation by the control compound.
36. **(Original)** The method of claim 28, wherein the translocation of the TMR is measured by determining the localization in the plasma membrane, pits, endosomes, endocytic vesicles, or cytosol.
37. **(Original)** The method of claim 29, wherein the GPCR is a class A, or class B receptor.
38. **(Original)** The method of claim 29, wherein the GPCR is a μ opioid, R1AR, f32AR, or dopamine receptor.
39. **(Original)** The method of claim 30, wherein the translocation of the TMR is measured by visualization of a radioisotope, an epitope tag, an affinity label, an enzyme, a fluorescent group, or a chemiluminescent group attached to the arrestin or the TMR.
40. **(Original)** The method of claim 28, wherein the signaling is measured at the same time as the translocation is measured.
41. **(Original)** The method of claim 28, wherein the cell is exposed to the compound once, and wherein the cell is not exposed a second time to the compound.
42. **(Withdrawn)** A compound discovered by the method of claim 28.
43. **(Original)** The method of claim 28, wherein the TMR is a rat, human, mouse, pig, or primate TMR.
44. **(Original)** The method of claim 28, wherein the method is repeated, and wherein the TMR in the repeat method is from a different species than in the original method.
45. **(Original)** The method of claim 44, wherein a test compound that is a TMRA in the original method is not a TMRA in the repeat method, and wherein the repeat method contains a TMR from a different species.
46. **(Original)** The method of claim 28, wherein the test compound is from a combinatorial library.

47. **(Original)** The method of claim 28, wherein the signaling in the presence of the test compound is approximately equal to or greater than the signaling in the presence of the control compound.
48. **(Original)** The method of claim 28, wherein the method is repeated at different concentrations of compound to yield a dose response curve for the signaling measurement and a dose response curve for the translocation measurement in the presence of the test compound.
49. **(Original)** The method of claim 48, wherein the quantitative determination includes a comparison of the dose response curve for the signaling measurement to the dose response curve for the translocation measurement.
50. **(Original)** The method of claim 48, wherein a second dose response curve for the signaling measurement and a second dose response curve for the translocation measurement are determined in the presence of control compound.
51. **(Original)** The method of claim 50, wherein dose response curve for the translocation measurement in the presence of the test compound is reduced as compared to the dose response curve for the translocation measurement in the presence of the control compound.
52. **(Original)** The method of claim 50, wherein the dose response curve for the signaling measurement in the presence of the test compound is approximately equal to or greater than the dose response curve for the signaling measurement in the presence of the control compound.
53. **(Original)** The method of claim 51, wherein the reduced translocation is determined by a decrease in the Max of the dose response curve for the translocation measurement in the presence of the test compound, as compared to the Max of the dose response curve for the translocation measurement in the presence of the control compound.
54. **(Original)** The method of claim 51, wherein the reduced translocation is determined by an increase in the EC50 of the dose response curve for the translocation measurement in the presence of the test compound, as compared to the EC50 of the dose response curve for the translocation measurement in the presence of the control compound.

55. **(Original)** A method of identifying a transmembrane receptor (TMR) agonist, wherein the TMR agonist (TMRA) is capable of activating TMR signaling while exhibiting reduced TMR internalization over a control compound, comprising the steps of:

(a) providing a cell comprising at least one TMR, or a biologically active fragment thereof, wherein the cell further comprises arrestin, or a biologically active fragment thereof,

(b) exposing the cell to at least one test compound,

(c) measuring the signaling at one or more concentration of compound,

(d) measuring the translocation of the TMR at one or more concentration of compound, and

(e) quantitatively determining if the internalization is reduced by comparing the TMR internalization in the presence of the test compound to the TMR internalization in the presence of a control compound, and wherein the signaling is activated as compared to TMR signaling in the absence of agonist.

56. **(Original)** The method of claim 55, wherein the TMR is a GPCR.

57. **(Original)** The method of claim 55, wherein the translocation of the TMR is measured by monitoring localization of a detectable molecule bound to the arrestin or the TMR.

58. **(Original)** The method of claim 55, wherein signaling is quantitated by measuring an intracellular effector, wherein said effector may be cAMP, cyclic GMP, Calcium, a lipid, phosphatidylinositol, a hydrogen ion, or an ion transport molecule.

59. **(Original)** The method of claim 55, wherein the control compound is a natural ligand or natural agonist.

60. **(Original)** The method of claim 55, wherein the test compound is a modified natural ligand or modified natural agonist.

61. **(Original)** The method of claim 55, wherein the test compound is a known pharmaceutically relevant compound, or is derived from a known pharmaceutically relevant compound.

62. **(Original)** The method of claim 55, wherein the signaling is activated for a longer time period after stimulation by the TMRA than the length of time of activation after stimulation by the control compound.
63. **(Original)** The method of claim 55, wherein the translocation of the TMR is measured by determining the localization in the plasma membrane, pits, endosomes, endocytic vesicles, or cytosol.
64. **(Original)** The method of claim 56, wherein the GPCR is a class A, or class B receptor.
65. **(Original)** The method of claim 56, wherein the GPCR is a μ opioid, $\sim 3_1$ AR, $\sim 3_2$ AR, or dopamine receptor.
66. **(Original)** The method of claim 55, wherein the translocation of the TMR is measured by visualization of a radioisotope, an epitope tag, an affinity label, an enzyme, a fluorescent group, or a chemiluminescent group attached to the arrestin or the TMR.
67. **(Original)** The method of claim 55, wherein the signaling is measured at the same time as the translocation is measured.
68. **(Original)** The method of claim 55, wherein the cell is exposed to the compound once, and wherein the cell is not exposed a second time to the compound.
69. **(Withdrawn)** A compound discovered by the method of claim 55.
70. **(Original)** The method of claim 55, wherein the TMR is a rat, human, mouse, pig, or primate TMR.
71. **(Original)** The method of claim 55, wherein the method is repeated, and wherein the TMR in the repeat method is from a different species than in the original method.
72. **(Original)** The method of claim 71, wherein a test compound that is a TMRA in the original method is not a TMRA in the repeat method, and wherein the repeat method contains a TMR from a different species.
73. **(Original)** The method of claim 55, wherein the test compound is from a combinatorial library.

74. **(Original)** The method of claim 55, wherein the signaling in the presence of the test compound is approximately equal to or greater than the signaling in the presence of the control compound.
75. **(Original)** The method of claim 55, wherein the method is repeated at different concentrations of compound to yield a dose response curve for the signaling measurement and a dose response curve for the translocation measurement in the presence of the test compound.
76. **(Original)** The method of claim 75, wherein the quantitative determination includes a comparison of the dose response curve for the signaling measurement to the dose response curve for the translocation measurement.
77. **(Original)** The method of claim 75, wherein a second dose response curve for the signaling measurement and a second dose response curve for the translocation measurement are determined in the presence of control compound.
78. **(Original)** The method of claim 77, wherein dose response curve for the translocation measurement in the presence of the test compound is reduced as compared to the dose response curve for the translocation measurement in the presence of, the control compound.
79. **(Original)** The method of claim 77, wherein the dose response curve for the signaling measurement in the presence of the test compound is approximately equal to or greater than the dose response curve for the signaling measurement in the presence of the control compound.
80. **(Original)** The method of claim 78, wherein the reduced translocation is determined by a decrease in the Max of the dose response curve for the translocation measurement in the presence of the test compound, as compared to the Max of the dose response curve for the translocation measurement in the presence of the control compound.
81. **(Original)** The method of claim 78, wherein the reduced translocation is determined by an increase in the EC50 of the dose response curve for the translocation measurement in the presence of the test compound, as compared to the EC50 of the dose response curve for the translocation measurement in the presence of the control compound.
82. **(Original)** A method of identifying a modified TMR agonist, wherein the modified TMRA is capable of activating TMR signaling while exhibiting reduced TMR internalization over an unmodified compound, comprising the steps of

- (a) providing an agonist or ligand of a TMR,
- (b) modifying the agonist or ligand,
- (c) providing a cell comprising at least one TMR, or a biologically active fragment thereof, wherein the cell further comprises arrestin, or a biologically active fragment thereof,
- (d) exposing the cell to a modified agonist(s) or modified ligand(s),
- (e) measuring the signaling at two or more concentrations of compound,
- (f) measuring the translocation of the TMR at two or more concentrations of compound, and
- (g) quantitatively determining if the internalization is reduced by comparing the TMR internalization in the presence of the test compound to the TMR internalization in the presence of an unmodified compound, and wherein the signaling is activated as compared to TMR signaling in the absence of agonist.

83. **(Original)** The method of claim 82, wherein the TMR is a GPCR.

84. **(Original)** The method of claim 82, wherein the translocation of the TMR is measured by monitoring localization of a detectable molecule bound to the arrestin or the TMR.

85. **(Original)** The method of claim 82, wherein signaling is quantitated by measuring an intracellular effector, wherein said effector may be cAMP, cyclic GMP, Calcium, a lipid, phosphatidylinositol, a hydrogen ion, or an ion transport molecule.

86. **(Original)** The method of claim 82, wherein the control compound is a natural ligand or natural agonist.

87. **(Original)** The method of claim 86, wherein the natural ligand or natural agonist is a known pharmaceutically relevant compound, or is derived from a known pharmaceutically relevant compound.

88. **(Withdrawn)** A compound discovered by the method of claim 82.

89. **(Original)** The method of claim 82, wherein the signaling is activated for a longer time period after stimulation by the TMRA than the length of time of activation after stimulation by the control compound.

90. **(Original)** The method of claim 82, wherein the translocation of the TMR is measured by determining the localization in the plasma membrane, pits, endosomes, endocytic vesicles, or cytosol.
91. **(Original)** The method of claim 83, wherein the GPCR is a class A, or class B receptor.
92. **(Original)** The method of claim 83, wherein the GPCR is a μ opioid, κ AR, δ AR, or dopamine receptor.
93. **(Original)** The method of claim 84, wherein the translocation of the TMR is measured by visualization of a radioisotope, an epitope tag, an affinity label, an enzyme, a fluorescent group, or a chemiluminescent group attached to the arrestin or the TMR.
94. **(Original)** The method of claim 82, wherein the signaling is measured at the same time as the translocation is measured.
95. **(Original)** The method of claim 82, wherein the cell is exposed to the compound once, and wherein the cell is not exposed a second time to the compound.
96. **(Withdrawn)** A compound discovered by the method of claim 82.
97. **(Original)** The method of claim 82, wherein the TMR is a rat, human, mouse, pig, or primate TMR.
98. **(Original)** The method of claim 82, wherein the method is repeated, and wherein the TMR in the repeat method is from a different species than in the original method.
99. **(Original)** The method of claim 98, wherein a test compound that is a TMRA in the original method is not a TMRA in the repeat method, and wherein the repeat method contains a TMR from a different species.
100. **(Original)** The method of claim 82, wherein the test compound is from a combinatorial library.
101. **(Original)** The method of claim 82, wherein the signaling in the presence of the test compound is approximately equal to or greater than the signaling in the presence of the control compound.

102. **(Original)** The method of claim 82, wherein the method is repeated at different concentrations of compound to yield a dose response curve for the signaling measurement and a dose response curve for the translocation measurement in the presence of the test compound.
103. **(Original)** The method of claim 102, wherein the quantitative determination includes a comparison of the dose response curve for the, signaling measurement to the dose response curve for the translocation measurement.
104. **(Original)** The method of claim 102, wherein a second dose response curve for the signaling measurement and a second dose response curve for the translocation measurement are determined in the presence of control compound.
105. **(Original)** The method of claim 104, wherein dose response curve for the translocation measurement in the presence of the test compound is reduced as compared to the dose response curve for the translocation measurement in the presence of the control compound.
106. **(Original)** The method of claim 104, wherein the dose response curve for the signaling measurement in the presence of the test compound is approximately equal to or greater than the dose response curve for the signaling measurement in the presence of the control compound.
107. **(Original)** The method of claim 105, wherein the reduced translocation is determined by a decrease in the Max of the dose response curve for the translocation measurement in the presence of the test compound, as compared to the Max of the dose response curve for the translocation measurement in the presence of the control compound.
108. **(Original)** The method of claim 105, wherein the reduced translocation is determined by an increase in the EC50 of the dose response curve for the translocation measurement in the presence of the test compound, as compared to the EC50 of the dose response curve for the translocation measurement in the presence of the control compound.